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FURTHER STUDIES UPON THE RELATION OF THE  
PSEUDO-DIPHTHERITIC BACILLUS TO THE  
DIPHTHERITIC BACILLUS.

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## FURTHER STUDIES UPON THE RELATION OF THE PSEUDO-DIPHTHERITIC BACILLUS TO THE DIPHTHERITIC BACILLUS.

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In a communication recently made by Professor Welch and myself, the results of our bacteriological examination of a series of cases of diphtheria and the reasons for regarding the Klebs-Löffler bacillus as a specific cause of genuine, primary diphtheria, were presented.<sup>1</sup> In this paper we called attention to the unsatisfactory status of the question regarding the so-called pseudo-diphtheritic bacillus. The present communication is intended to shed some light upon this unsettled question.

While there is a general agreement of opinion as to the morphological, cultural, and pathogenic properties of the genuine virulent bacillus diphtheriae, there is no little confusion as to the nature of the so-called pseudo-diphtheritic bacillus and in general as to the nature of bacilli found in the throat, which, although resembling in many respects the diphtheritic bacillus, still differ from it in some important particulars.

Löffler<sup>2</sup> was the first to use the term pseudo-diphtheritic bacillus, and to call attention to the properties which he considered to differentiate it from the genuine diphtheritic bacillus. By means of agar-plate cultures made from a growth on blood-serum obtained from a case of diphtheria, he isolated two sorts of bacilli, the one corresponding in all respects to the genuine diphtheritic bacillus, the other differing from this organism in the following respects: it was devoid of pathogenic properties for guinea-pigs, its colonies were less distinctly notched at the margins and were whiter than those of the virulent diphtheritic bacillus, it was somewhat smaller than the virulent bacillus, and its ends less frequently swollen. He calls attention to the possibility that there may be several species of bacilli belonging to the same group as the virulent diphtheritic bacillus, as there are several species belonging to the group of the cholera bacillus, and that careful observation of the morphological and biological peculiarities will enable us to distinguish the genuine diphtheritic bacillus from the pseudo-diphther-

<sup>1</sup> Welch and Abbott, *The Johns Hopkins Hospital Bulletin*, 1891, No. 11.

<sup>2</sup> Löffler, *Centralblatt f. Bacteriologie u. Parasitenkunde*, 1887, Band II, p. 105.



ritic just as we can distinguish the cholera bacillus from other similar species of bacteria.

In 1887 and 1888 von Hoffmann,<sup>1</sup> from the examination of over 60 cases of various kinds, including genuine diphtheria, diphtheritis faucium accompanying scarlatina and measles, simple pharyngeal catarrh, and normal mucous membranes, came to the conclusion that a bacillus resembling the Klebs-Löffler bacillus closely in its morphological and cultural properties but differing from it in certain respects, is a very common, probably regular, inhabitant of the pharynx. This bacillus von Hoffmann identified with the one already described by Löffler as the pseudo-diphtheritic bacillus, and to the differential characters already given by Löffler he added another, viz: that the virulent diphtheritic bacillus grows feebly upon plain nutrient agar, whereas the pseudo-diphtheritic bacillus grows luxuriantly upon this medium, and that, too, at the ordinary temperatures of the room. Von Hoffmann, furthermore, found in diphtheria and other conditions a bacillus indistinguishable from the genuine diphtheritic bacillus in its morphology and behavior on culture media, but devoid of pathogenic properties. In one case of measles and in one case without any affection of the larynx or pharynx, he found a bacillus indistinguishable from the virulent diphtheritic bacillus.

In 1888 Babes<sup>2</sup> published an article, *Ueber isolirt färbbare Antheile von Bakterien*, in which he described various species of bacteria which resemble more or less closely, especially as regards the presence of stained granules in them, the Klebs-Löffler bacilli. Upon the basis especially of this peculiarity and of the property of presenting swollen ends and segments, he distinguished a family group of bacteria, to which belong the Löffler bacillus, the Xerosis bacillus, a bacillus found by him in three cases of trachoma, and also bacilli found by him in the margin of a perforating syphilitic ulcer of the palate and in the spleen and lungs of the same case, and in a case of genuine lobar pneumonia. In a phlegmonous inflammation of the neck, resulting from genuine diphtheria, Babes found a bacillus resembling the Löffler bacillus, but larger and presenting large terminal oval spores. It is evident that in this family group Babes includes bacilli which are readily distinguishable from the Löffler bacillus, as well as some which closely resemble the latter organism.

Orthmann<sup>3</sup> (1889), in a case of apparently diphtheritic affection of the mucous membrane of the cheek, and in a case of purulent meningitis of the convexity, in the exudate and in the nose, isolated a bacillus which he found to be identical with the pseudo-diphtheritic bacillus of Löffler and von Hoffmann, and he confirmed the statements of these observers as to the differential characters of this bacillus.

Kolisko and Paltauf<sup>4</sup> (1889) were able to find the pseudo-diphtheritic bacillus of von Hoffmann only very rarely. They confirmed the statement

<sup>1</sup> Von Hoffmann, *Tagebl. d. 60. Versamml. Deutscher Naturf. u. Aerzte in Wiesbaden*, 1887, p. 119, and *Wiener Med. Wochenschrift*, 1888, No. 3 u. 4. (Quoted from Baumgarten's *Jahresbericht*, 1887, p. 245.

<sup>2</sup> Babes, *Zeitschrift f. Hygiene*, Bd. V., p. 173.

<sup>3</sup> Orthmann, *Berliner Klin. Wochenschrift*, 1889, No. 10, p. 218.

<sup>4</sup> Kolisko and Paltauf, *Wiener Klin. Wochenschrift*, 1889, No. 8.

that this bacillus grows very well upon gelatine and especially upon agar-agar, whereas the genuine diphtheritic bacillus grows feebly on these media, which therefore furnish a useful diagnostic means for distinguishing the two species.

Zarniko<sup>1</sup> (1889) obtained results contradictory in many important details to those of von Hoffmann. He found that the diphtheritic bacillus grows well upon gelatine (as had been previously noted by Orthmann) and upon agar-agar (as had been observed by Flügge). In 29 cases in which the healthy or the catarrhal mucous membrane of the pharynx was examined, he failed to find in a single instance the genuine diphtheritic bacillus, and in only one instance did he find a bacillus which he identified with the pseudo-diphtheritic bacillus. He describes the following characteristics of the pseudo-diphtheritic bacillus: The growth upon Löffler's blood-serum is indistinguishable from that of the diphtheritic bacillus and its temperature limits are the same. On gelatine and upon agar-agar it grows more luxuriantly on the surface, so that glistening white deposits appear in distinction to the delicate grayish-white deposits of the diphtheritic bacillus. A fundamental difference is found in the growth of the two organisms in bouillon. The pseudo-diphtheritic bacillus renders the bouillon cloudy from the third day, and the sediment is more compact and white than in the case of the genuine bacillus. The bouillon retains its alkaline reaction, whereas the diphtheritic bacillus in a short time produces an acid reaction. The rods are somewhat shorter and plumper, and involution and degeneration forms are less common than in the case of the diphtheritic bacillus. It is devoid of pathogenic properties for guinea-pigs, rabbits and white mice. Zarniko found the diphtheritic bacillus to be in all cases pathogenic for guinea-pigs.

Escherich<sup>2</sup> (1890) found the pseudo-diphtheritic bacillus only twice and infers that the circumstances of von Hoffmann's investigations must have been of a very unusual character. Escherich bases the distinction between the pseudo-diphtheritic bacillus and the diphtheritic bacillus upon characters noted by previous observers, viz.: for the former, the pure white color of the colonies, the richer growth upon agar, the cloudiness and permanently alkaline reaction of the bouillon, and the microscopic examination. These differential characters he considers sufficient for the diagnosis. He says that in no case did he find bacilli morphologically and in cultures identical with the Löffler bacillus, but devoid of pathogenic properties when tested upon animals.

Klein<sup>3</sup> (1890) found in the examination of 22 cases of diphtheria two species of bacilli, the one, found in all cases, doubtless identical with the Löffler bacillus, the other, found in 12 cases, probably, he thinks in his latest publication, identical with the pseudo-diphtheritic bacillus.

His description of both of these organisms can not be reconciled altogether with the statements of other observers. The bacillus found only in 12

<sup>1</sup> Zarniko, *Cent. f. Bacter. u. Parasitenk.*, 1889, Bd. VI, p. 227.

<sup>2</sup> Escherich, *Cent. f. Bacteriol. u. Parasitenk.*, 1890, Bd. VII, p. 11.

<sup>3</sup> Klein, *Ibid.*, Bd. VII, Nos. 16 and 17, and *Nineteenth Annual Report of the Local Government Board for 1889-90*. Supplement containing the *Report of the Medical Officer for 1889*, p. 143.

cases, which he at first was inclined to identify with the Löffler bacillus, but which he now considers as the pseudo-diphtheritic bacillus, was found to be non-pathogenic for guinea-pigs, except in two instances, in which the autopsies showed entirely different appearances from those in animals inoculated with the diphtheritic bacillus. Klein's pseudo-diphtheritic bacillus does not grow at a temperature below 20° C.; it does not grow on potato; its colonies on gelatine and on agar resemble closely those of the genuine diphtheritic bacillus, which, however, is distinguishable by growing well at a temperature of 19°-20° C. and developing even at 16° C.; it produces in alkaline bouillon at 37° C. only slight cloudiness and forms flakes and granules, whereas his genuine diphtheritic bacillus grows more rapidly in bouillon, forming, after 24 hours, strong uniform turbidity; its cultures die out in a few months, whereas cultures of the true diphtheria bacillus survive at room-temperature for over 18 months; it presents fewer so-called degeneration and involution forms than the true bacillus. It will be observed that Klein's statement as to the growth of the two species in bouillon differs from that of Zarniko, and that he does not describe the characteristic growth of the true diphtheritic bacillus in bouillon. A culture of Klein's true bacillus of diphtheria examined by Löffler, was pronounced by him to be the genuine bacillus diphtheriae.

Spronck<sup>1</sup> (1890) found in only one out of 13 cases of diphtheria, a non-virulent bacillus resembling the Löffler bacillus. Apparently solely on the ground of absence of virulence he designates this as the pseudo-diphtheritic bacillus. There were found in the same case and in all the other cases virulent diphtheritic bacilli.

Beck<sup>2</sup> (1890) says that he found the pseudo-diphtheritic bacillus in a large number of cases of diphtheria together with the virulent bacillus. In 66 healthy children the pseudo-diphtheritic bacillus was found 22 times and in 64 children with various non-diphtheritic affections of the mouth and throat it was found 14 times. In none of the latter 130 children was the virulent Löffler bacillus found once. The differential characters are essentially those given by Löffler, viz.: besides absence of virulence, the pseudo-diphtheritic bacillus grows on blood-serum with more of a yellowish tint, on agar-agar much more rapidly and with colonies presenting more uniform, less notched margins than is the case with the genuine bacillus. Beck speaks of the color of these colonies as lighter ("heller") than that of the diphtheritic colonies. The rods are somewhat thicker than the Löffler bacilli but like these they vary in length.

Roux and Yersin<sup>3</sup> (1890) have made the most elaborate study of the relation between the virulent diphtheritic bacillus and the non-virulent forms.

Differences in the virulence of the Löffler bacillus as tested upon animals had been noted to some extent by previous investigators, especially by Brieger and Fraenkel.<sup>4</sup> Roux and Yersin examined 100 cases of diphtheria

<sup>1</sup> Spronck, *Centralbl. f. allg. Path. u. Patholog. Anat.*, 1890, Bd. I, p. 218.

<sup>2</sup> Beck, *Zeit. f. Hyg.*, Bd. VIII, p. 434.

<sup>3</sup> Roux and Yersin, *Annales de l'Institut Pasteur*, 1890, Tome IV, p. 409.

<sup>4</sup> Brieger and Fraenkel, *Berliner Klin. Wochenschrift*, 1890, No. 11.

in all of which they found the Löffler bacillus. Cultures obtained from 40 out of 53 fatal cases were inoculated into guinea pigs, all of which succumbed in a period of time varying from less than 24 hours to four days. Cultures from 39 out of 47 cases which recovered, when tested upon guinea pigs, gave the following results: 17 of the cultures killed the animals in less than three days, seven in from four to nine days, five killed only some of the inoculated animals and ten produced only oedema varying from severe oedema followed by an eschar to a slight, quickly disappearing oedema, from which the animal recovered. They found the attenuated bacillus together with the virulent form less frequently in the fatal than in the mild cases and they noted in severe cases terminating in recovery a tendency for the attenuated bacilli to take the place of the virulent bacilli towards the end of the disease. They found the non-virulent, so-called pseudo-diphtheritic bacillus in the pharynx of 15 out of 45 children examined in the Hôpital des Enfants Malades in Paris and in 26 out of 59 children in a healthy village on the sea-coast where no diphtheria had occurred for a long time. The same bacillus was found once in ten of the attendants in the pavilion for diphtheria, twice in six children affected with simple angina and five times in seven cases of measles. In all the non-diphtheritic cases the pseudo-diphtheritic bacilli were very scanty, rarely more than one to four colonies being present in a tube, and often only one colony in several tubes inoculated with mucus from the throat, so that they do not consider that the presence of the pseudo-diphtheritic bacillus causes any serious difficulty in the diagnosis of diphtheria by culture methods which show a large number of colonies of the specific bacillus in true diphtheria. Measles seem to offer an especially favorable soil for the development of the pseudo-diphtheritic bacillus. The only distinction which Roux and Yersin regard as of any weight between the so-called pseudo-diphtheritic bacillus and the virulent diphtheritic bacillus, is the absence of virulence in the former. The colonies of both are identical on blood-serum, but the pseudo-diphtheritic bacillus is often shorter in colonies on this medium. The pseudo-diphtheritic bacillus grows usually, but not invariably more luxuriantly in bouillon, especially at the temperature of 20°-22° C., and produces a more voluminous deposit than is the case with the virulent bacillus. The changes in reaction of the bouillon from alkaline to acid, and then back to alkaline, occurs more quickly with the pseudo-diphtheritic form. In contradistinction from the aerobic growth, the development of the pseudo-diphtheritic bacillus in a vacuum is less abundant than that of the virulent species. The pseudo-diphtheritic bacillus does not produce toxic proteids in its cultures, and when inoculated subcutaneously into guinea-pigs, it is incapable of killing the animals, although it may cause sometimes more or less local oedema. As regards the question whether the so-called pseudo-diphtheritic bacillus and the virulent diphtheritic bacillus are two distinct species of bacteria, or whether the one is simply an attenuated form of the other, Roux and Yersin express themselves in favor of the latter view for the following reasons: the mere absence of virulence is not sufficient ground for separating one organism from another as a distinct species, and all alleged morphological and cultural differences between the pseudo-diphtheritic and

the diphtheritic bacillus are too feeble and inconstant to distinguish them; all degrees of virulence exist between that capable of killing guinea-pigs in less than 24 hours to that causing only slight, temporary œdema at the point of inoculation; the gradual replacement of the virulent bacillus by one less virulent or completely attenuated as a case of diphtheria progresses toward recovery, speaks in favor of the doctrine of attenuation; the virulent diphtheritic bacillus can, although with much difficulty, be attenuated by a peculiar procedure outside of the body, so as to resemble the pseudo-diphtheritic bacillus, and finally diphtheritic bacilli so attenuated as to produce only local œdema without killing the animal may, by a combined inoculation with the *erysipelas strepto-coccus*, be made to acquire increased virulence so as to destroy guinea-pigs by inoculation. This transformation, however, has not been accomplished with the pseudo-diphtheritic bacillus devoid of all virulence, and until this has been done Roux and Yersin do not consider that the view which they advocate is fully proven.

It is apparent from the foregoing review of the literature of our subject that further investigations are needed to determine the nature of the so-called pseudo-diphtheritic bacillus or bacilli, and that the statements of various writers are conflicting. It is established that bacilli, with all the characters of the virulent Löffler bacillus, are to be found only very exceptionally in conditions where diphtheria is absent. In diphtheria several observers have found, together with genuine diphtheritic bacilli, bacilli resembling the Löffler bacillus, but devoid of virulence. Many have failed to find in the series of cases examined by them these non-virulent bacilli, others have found them very rarely, while von Hoffmann, and Roux and Yersin have obtained them not so very infrequently. Most of the German investigators have followed Löffler in considering these non-virulent bacilli found in diphtheria as a species distinct from the virulent diphtheritic bacilli, the distinction being based primarily upon the absence of virulence, but also upon slight morphological and cultural differences, the chief of which being more luxuriant and whiter growth upon agar-agar and capability of growth at lower temperatures. To these distinctions Zarniko adds the different modes of growth of the two organisms in bouillon. In general, the descriptions given of the non-virulent bacilli are unsatisfactory, and many observers seem to have gone little further than to determine the absence of virulence, and solely on this ground have designated the bacilli in question as pseudo-diphtheritic. As already mentioned, Klein's pseudo-diphtheritic bacillus can not, from his description, be identified with the pseudo-diphtheritic bacillus of Löffler and of von Hoffmann. Roux and Yersin consider that the morphological and cultural differences between the diphtheritic and the pseudo-diphtheritic bacillus are inconstant and when present insufficient to establish different species. They expressly state that they have found in diphtheria bacilli absolutely indistinguishable from the Löffler bacilli, except by the absence of pathogenic properties when tested on animals.

It must be admitted that the morphological and cultural differences found by Löffler, von Hoffmann, and Zarniko to exist between the diphtheritic and the pseudo-diphtheritic bacilli lose much of their value by the

variability in the properties of the virulent Löfller bacillus. This bacillus varies in size, in the readiness with which it grows at ordinary temperatures on gelatine and on agar-agar, in the luxuriance of its growth on these media and in the mode of its growth in bouillon which may be rendered diffusely cloudy instead of remaining clear with clumps at the sides and bottom of the tube.

Whereas the majority of investigators have found the pseudo-diphtheritic bacillus only rarely, von Hoffmann, Beck and Roux and Yersin, who have examined the largest number of cases, have found it not infrequently in healthy individuals, or in those with non-diphtheritic affections, so that it would seem to be, at least in some places a common inhabitant of the mouth and throat, although hardly a "regelmässiger Bewohner" of the pharynx as claimed by von Hoffmann. No differences have been determined between the pseudo-diphtheritic bacillus found in some cases of diphtheria and that observed in other conditions, but upon this as upon many other points concerning the pseudo-diphtheritic bacillus, statements are often meagre or indefinite. It seems that the term pseudo-diphtheritic bacillus is now used loosely to designate a group of bacilli which resemble the Löfller bacillus, but which are without pathogenic properties when tested on guinea-pigs. The resemblance in morphology and cultural properties to the Löfller bacillus may amount to apparent identity, or there may be marked differences. It can hardly be otherwise than that such a loose usage must cover a variety of organisms, and that, therefore, the term itself must lack definite application.

Since the publication of the work upon the etiology of diphtheria by Prof. Welch and myself, I have devoted my time to the study of a number of cases of ordinary benign affections of the throat. These cases presented very slight constitutional symptoms, in fact, with two exceptions, were from the patients who daily visit the dispensary of the Johns Hopkins Hospital.

These studies were made, in all, upon 53 patients, 9 of whom were suffering from acute pharyngitis, 14 from acute follicular tonsillitis, 8 from ordinary post-nasal catarrh, 2 from simple enlarged tonsils, 15 from chronic pharyngitis, 1 from subacute laryngitis, 1 from chronic laryngitis, 1 from rhinitis, and 2 from an affection of the tonsils and pharynx which rendered a diagnosis without bacteriological aid more or less difficult.<sup>1</sup> The last two cases were described by the writer in a paper before the Johns Hopkins Hospital Medical Society, May 18th, 1891. They are inserted here for the purpose of completing the group.

For the examination of these 53 cases of various affections of the throat, the blood-serum mixture of Löfller was employed. The inoculations were conducted in the way common to this work.

In 49 of the cases of this group nothing of particular interest was observed. The tubes showed a variety of organisms, some of which are well known, particularly the pyogenic cocci, while others could not readily be identified.

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<sup>1</sup> I am indebted to Doctor Wm. Sidney Thayer, Resident Physician at the Johns Hopkins Hospital, for his valuable assistance in obtaining these cases for me.

As they bore no immediate relations to the work under consideration they were dispensed with as soon as it was established that they were in no way to be confounded with the organism of diphtheria.

In four of these cases now to be described were found organisms which in their morphology and cultural peculiarities were so like the genuine bacillus diphtherie of Löffler that they were subjected to detailed study. The clinical histories of the cases and description of the organisms found in them are as follows:—

CASE I.—J. B., aged 59 years, was admitted to the Johns Hopkins Hospital, March 19th, 1891, suffering from shortness of breath and moderate dropsy of the legs. Examination revealed hypertrophy of the heart with high-tension pulse, albuminuria and slight oedema of the lungs. At the end of two weeks he had improved considerably, but complained of slight fever and sore throat. His voice was husky and thick and he had some difficulty and pain in swallowing food. The pillars of the fauces, the uvula and tonsils were swollen and oedematous, of an intense crimson-red color and covered with a thin, grayish-white, slightly adherent material. There was a moderate elevation of temperature, not exceeding 102° F.

He was immediately isolated.

The soreness of the throat was of short duration, and in five days every vestige of the thin membranous-looking deposit had disappeared, no extension whatever having occurred during this time. He was discharged from the Hospital on April 18th, 1891.

CASE II.—P. K. was well up to Sunday, April 19th. At 3 p. m., April 19th, he began to feel badly. There was a slight chill, no shaking, fever, slept badly. He complained of sore throat and pain upon swallowing. He had considerable headache and pain through the limbs and back. There was no bleeding from the nose, no cough, no vomiting. Bowels rather constipated. His temperature on admission to the ward was 104.8° F., pulse 112, full and bounding. Respiration quick, skin hot and moist, tongue clean and dry, face flushed.

Uvula, tonsils and pillars of the fauces injected and red and covered with a yellow, pultaceous deposit.

April 21st (the day following admission), temperature 100° F., pulse 100. Patient feels much better. He has had aconite and citrate of potash every 3 hours.

April 27th. Throat still sore—a grayish-white deposit over tonsils and pillars of fauces and soft palate.

April 29th. Patient discharged well.

Microscopic examination of cover-slip preparations from the deposit in the throat of each of these patients revealed the presence of a variety of organisms, among which could be made out the irregular curved, clubbed and spindle-forms common to the bacillus of Löffler. It may be said here that it is not safe to rely upon the microscopic examination alone of the smear preparations from these deposits, as there exist in the mouth, and particularly in the mouths of those having decayed teeth, curved and spindle-shaped bodies, the morphology of which is so like that of some of

the forms common to the bacillus of Löfller that they might easily be mistaken for this organism.

In Case I cultures were made from the thin membranous deposit, the blood-serum mixture of Löfller being employed for the purpose. After 24 hours at 37° C., the tubes presented the appearance common to those made from an undoubted case, except, perhaps, there was an unusual number of colonies of the staphylococcus pyogenes aureus. From these blood-serum tubes there was but little difficulty in obtaining in pure culture an organism which in all of its morphological and cultural peculiarities was identical with the bacillus of Löfller, with a virulent culture of which it was constantly compared.

The colonies of this organism on nutrient glycerine agar-agar could not be distinguished from those resulting from the growth of the true organism of diphtheria.

In bouillon it presented the characteristic sedimentation and clumping without producing a diffuse clouding of the upper layers of the fluid.

On gelatine it did not produce liquefaction and its colonies were of the characteristic form.

It is non-motile.

On potato it grew in the way common to the bacillus of Löfller, as has been pointed out in the paper by Professor Welch and myself, as an invisible layer quite similar to that described for the bacillus of typhoid fever.

In short, it was not possible to differentiate by any of the culture methods at our disposal, between this organism and that described by Löfller.

The results, however, were different when this organism was inoculated into the subcutaneous tissues of guinea-pigs and kittens, both of which possess a marked susceptibility to the action of the *b. diphtheriae* of Löfller.

Repeated inoculations of cultures of this organism growing on different media were negative, whereas the control animals inoculated with the cultures of the true diphtheria bacillus were positive in every case, the tissue changes being in each instance identical with those commonly following such inoculations.

From Case II the cover slips likewise presented a variety of organisms, and as in Case I a number of these were of a more or less suspicious appearance.

The cultures from this case were upon glycerine agar-agar as there was at the time no blood-serum in the laboratory. After 24 hours at 37° C., the Esmarch tubes presented a striking appearance—they contained a moderate number of colonies of different appearance, the most conspicuous being those of a bright yellow color which proved to be made up of a coccus and a few others which presented an appearance which one might conceive to be that of the bacillus of diphtheria very much magnified. They were larger, denser, and much more moist and opaque than are those of the *b. diphtheriae*.

Microscopic examination, however, of cover-slip preparations from those colonies showed them to be made up of an organism, the morphology of which is strikingly like that of the organism of diphtheria. All of the different curved, pointed and spindle shapes were present as well as the

large bizarre and broken involution forms so common to the true bacillus of diphtheria.

On the whole, however, these organisms are slightly larger than those of Löffler.

The growth of this organism on gelatine, agar-agar and blood-serum is so like that of the bacillus of Löffler that, excepting a greater degree of luxuriance, it is hardly possible at all times to tell the one from the other.

In bouillon there is macroscopically a tendency toward a diffuse clouding of the medium, but if a drop of this culture is examined microscopically without drying (as a hanging drop) it will be seen that the tendency throughout is to grow in minute clumps.

This organism is non-motile.

The most striking difference between this organism and the usual Löffler bacillus is its behavior toward potato.

On potato it grows with moderate rapidity as a dry, dirty-brown layer which eventually covers nearly the whole surface of the potato. It is finely granular on the surface and irregularly lobulated around its borders.

This is an appearance that we have failed to find with the ordinary bacillus of Löffler and likewise is it absent from the organism which has been described for Case I.

Like the organism from Case I when inoculated into guinea pigs it has given us negative results, whereas the control animals inoculated at the same time with the true bacillus of diphtheria died with the characteristic lesions.

**CASE III.**—F. B., girl, age 11 years, came into the outpatient department of this Hospital on June 2d, complaining of sore throat. Was taken sick June 1st.

Examination reveals redness, injection and swelling of the tonsils. Small white plug in one of the crypts of left tonsil. Cervical lymphatics enlarged and painful. Temperature 99° F.

No evidence of a membrane anywhere to be found.

**Diagnosis.** Acute follicular tonsillitis.

**June 5th.** Child is reported well.

**June 6th.** Child is again seen. Examination reveals no trace of the acute process except a very minute white point over a crypt in left tonsil. Says she feels entirely well.

Cultures on Löffler's blood-serum mixture, from the plug on the left tonsil, from right tonsil and from the pharynx, shows after 48 hours at 38° C. (they were not examined after 24 hours) a number of large, dense, white, irregular and regular patches of growth which, upon microscopic examination, are seen to be made up of an organism, the morphology of which is strikingly suggestive of that of the bacillus of Löffler.

Other colonies composed of the small spindle-shaped bacilli, so frequently seen in many of the cases of true diphtheria studied by Prof. Welch and myself were also present. Here and there colonies of streptococci were to be found. The microscopic appearance of these tubes was similar to that of serum tubes from a case of true diphtheria, except, perhaps, the colonies

having the appearance of those growing from the bacillus of Löffler were somewhat fewer in number.

Microscopic examination of these large, creamy colonies showed them to be made up of bacilli the morphological peculiarities of which were identical with those of the Löffler bacillus. The spindle and clubbed forms, likewise the irregularly segmented rods, were everywhere present. Very conspicuous were the dark, almost black, points seen in the individual bacilli when stained with Löffler's methylene-blue solution.

From these colonies three sets of agar-agar Esmarch tubes were made. After about 19 hours at 37° C. there appeared upon the tubes colonies in every way identical with those of the true diphtheria bacillus when seen under similar circumstances. Microscopically, these colonies were seen to be made up of organisms which could not be distinguished morphologically from the organism of Löffler. Under low magnifying power these colonies, when upon the surface of the agar, were irregularly round or oval with more or less notched periphery. They were granular and possessed a dark, granular, ragged centre, surrounded by a lighter peripheral zone. They were very flat and were elevated but slightly above the surface of the medium. They are not glazed, but rather dull in their appearance when seen by reflected light. When deep down in the agar they are round or oval, coarsely granular and of a more or less bronze color. This growth is not so rapid as that of the organisms from Case II.

On potato its growth is best described as producing a slight diminution in the reflective properties of the surface of the potato. Of eight potatoes inoculated with this organism six showed an invisible growth, whereas on the remaining two there was a very fine granular deposit not easily to be seen with the naked eye. In short, the cultural peculiarities of the organism found in this case were identical with those of the organism found in true diphtheria.

It is not pathogenic for guinea-pigs or kittens.

CASE IV.—S. McS., male, adult. Clinical diagnosis of syphilitic pharyngitis. When he came to the dispensary the pharynx and both tonsils were injected. The tonsils slightly enlarged. No deposit anywhere in the throat.

Cultures from pharynx and tonsils gave a large number of colonies consisting of organisms which morphologically and in cultures are not distinguishable from the genuine diphtheritic bacillus. It sometimes clouds the bouillon and sometimes grows in the typical way in small macroscopic clumps. On blood-serum, agar-agar and gelatine it is not to be distinguished from the genuine diphtheria bacillus; it does not grow more luxuriantly, if anything less so than the Löffler bacillus. It grows invisibly on potato.

Two animals, guinea-pigs, were inoculated subcutaneously, each with one dose of a fresh culture on glycerine agar. The one culture was 24 hours, the other 48 hours old. These inoculations were made on June 27th and on July 13th. Neither animal presented any manifest effects from the inoculation, both having been kept under observation until August 7th, so that it is fair to assume that the organism is devoid of pathogenic properties.

From what we have seen in Cases I, III and IV, it is clear that there exists an organism indistinguishable from the genuine diphtheritic bacillus of Löffler, save by the absence of pathogenic properties.

The organism from Case II was easily distinguished from the bacillus of Löffler by its growth on potato, though in its morphology it was in many cases difficult to find any difference between the two.

From these observations we feel justified in agreeing with the opinion that has been advanced by other observers, particularly Hoffmann and Roux and Yersin, that under varying conditions the virulence of the true diphtheria bacillus may be observed to fluctuate in the degree of its intensity—at one time possessing the property in a high degree, at another presenting a decided attenuation and not unfrequently a complete absence of pathogenic power.

The organism obtained from Cases I, III and IV, we believe to be the true diphtheria bacillus which, from some unknown cause, has lost its virulence.

The organism obtained from Case II, though resembling closely the organism described by Löffler, presents a marked difference in its growth upon potato.

In the relation of these organisms to the ordinary staining reagents I can find no difference between them and the Löffler organism. They all stain readily by the Giemsa method and with the other dyes commonly employed in coloring bacteria. Their appearance when stained with Löffler's alkaline methylene-blue solution is identical. If one prepares coverslip preparations from cultures of the same age on the same medium, the one from a true diphtheria culture, the other from the organisms just described, the only difference that one can detect is that the bacillus from Case II, which grows visibly on potato and on all other media, presents a slightly coarser growth than is seen with the true diphtheria bacillus, is a little larger and presents occasionally more of the involution forms than does the Löffler organism.

The single point of distinction that can be made out between the organism obtained from Cases I, III, and IV and the true bacillus of diphtheria is in the absence of pathogenic properties from the former, whereas in addition to this point of distinction the organism from Case II gives, as has been stated, a decided and distinct growth upon the surface of sterilized potato, an appearance which one does not see with the Löffler organism and which has thus far been absent from the organism obtained from the other cases.

In a paper recently read before the Johns Hopkins Hospital Medical Society<sup>1</sup> I expressed the opinion that while the organism obtained from the faecal deposit in Case II had under all circumstances grown as a visible deposit on potato and the genuine bacillus diphtheriae of Löffler had in the great majority of cases given no naked-eye evidence of its growth on the same medium, still, as in the case of the typhoid bacillus, this apparent point

<sup>1</sup> "The Relation of the Pseudo-Diphtheritic Bacillus to the Diphtheritic Bacillus," by A. C. Albee, M.D., Assistant in Bacteriology and Hygiene, Johns Hopkins Hospital. *Johns Hopkins Hospital Bulletin*, No. 15, August, 1891.

of distinction may after subsequent work prove to be of more or less inconstancy and hence not so valuable a criterion for the differentiation of these organisms found in the faecal deposits as was at first supposed.

I have made a series of experiments with the view of shedding some light upon this subject:

From the single colonies on the Esmarch tubes made from the fauces of Case II six potatoes were inoculated. All grew *visibly* as a dirty brown, granular deposit. All were composed of organisms of the characteristic morphology of the genuine *b. diphtheriae*. From one of these potatoes a set of Esmarch tubes on nutrient agar-agar was made. Upon these tubes there appeared two kinds of colonies, but *both made up of morphologically similar organisms*. One set of these colonies were fine, blue-white, transparent, flat, dry, concentrically arranged and somewhat irregular around their borders—in short, they were in every way identical with those colonies growing from the true *b. diph.* The other colonies on this tube were cream-white, moist, somewhat elevated above the surface, tolerably regular in outline and, by transmitted light, indistinctly concentric in their arrangement.

From the *finest and most characteristic* colonies on the Esmarch tubes a set of Esmarch tubes was made, likewise a set from the *coarsest, cream-like colonies*. After 24 hours at 37° C. the appearance of the colonies on these two sets of Esmarch tubes were not only exactly alike, but they were in both cases identical with those growing from the ordinary virulent *b. diph.* of Löffler. After 72 to 96 hours, however, they had grown in both cases to an extent not commonly seen with the true *b. diphtheriae*.

After 48 hours' growth the colonies on both sets of Esmarch tubes showed differences. In the same tube, for example, from the same source, some of the colonies retained their fine, delicate, characteristic structure, while others showed a tendency to grow more luxuriantly, so that the details of the colonies became lost. Throughout the growth of all of these colonies they presented to the platinum needle a peculiar dry or mealy feel.

An effort was now made to determine if possible the significance of these differences in the growth of the colonies on these tubes. The first test to which they were subjected was the behavior toward potatoes.

From the Esmarch tubes made from the coarse creamy colonies on the Esmarch tubes from the original potato which showed the brown growth, the *coarsest* colony was selected and from it four potatoes were inoculated. After 24 hours at 37° C., all four potatoes showed a visible growth similar to that on the original potato. After 72 hours at 37° C., this growth has increased to such an extent that it resembles closely the growth of the *bact. coli commune* on potato under the same conditions. Microscopically these growths are composed of organisms of identically the morphology of the *b. diphtheriae*.

The *finest* colony on this set of Esmarch tubes was at the same time selected and from it four potatoes were inoculated. After 24 hours at 37° C., no growth was visible. After 72 hours, however, at the same temperature, all four of them showed distinct characteristic development of the same organism.

From the other series of Esmarch tubes made from the *fine* characteristic colonies on the Esmarch tubes from the original potato which showed the brown growth the *coarsest* colony was selected and from it four potatoes were inoculated. After 24 hours, one grew distinctly visible, one grew visibly and two invisibly. After 72 hours at 37° C., all four showed visible growth.

From the same tubes the *finest* colony was selected and from it four potatoes were inoculated. After 24 hours, three grew invisibly and one grew just visibly. After 72 hours all showed distinct visible growth but on some of the potatoes it was more extensive than on others.

In this series of experiments the potatoes were from the same source and were all prepared in the same way and at the same time. They were all inoculated at the same time.

From a *virulent* culture of the *b. diphtheriae* nine potatoes were inoculated. After 96 hours at 37° C., eight of these potatoes showed no visible growth, whereas one showed a growth which could just be made out upon its surface.

Reviewing the above experiments we find that in the majority of instances, if not quite constantly, the virulent organism is seen to grow invisibly on potato—now and then a very indistinct growth may be made out. Twice in a large number of inoculations upon potatoes have I seen it grow visibly, but even then its growth could only be seen with great difficulty. Among the descendants from the potato, from the original Esmarch tubes, from the patient (Case II), every grade of development upon potatoes under the same conditions are seen—from the very fine, almost invisible growth, up to the coarse growth, resembling that of the *bact. coli* commune.

Microscopically, no difference can be made out between the organisms comprising these potato cultures.

Whether these differences in the development of these organisms upon potato can be taken as indicating different degrees of departure from the pathogenic condition as seen in the genuine bacillus of Löffler, it is impossible to say as no regular or corresponding deviation in the periods of incubation of these different organisms in the bodies of animals could be made out, because, as has been stated, they were all without pathogenic properties.

During the course of this work efforts were made to determine if the inoculation of susceptible animals, with the cultures of these organisms which are so like the bacillus of Löffler, would have any effect in the production of immunity in these animals toward the subsequent inoculation of *virulent* cultures of the genuine *b. diphtheriae*.

Though the work can hardly be said to have been conducted on a very large scale, still the evidence obtained in these experiments does not confirm such an opinion.

Variations in the length of time which ensued between the time of inoculation and death of these animals, which were first treated with the non-virulent and subsequently with the virulent cultures, could be seen, but they were too slight in their degree to lead one to suppose that they were due to any protection afforded to the animal by the non-virulent cultures with which it had been inoculated.







